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Post-thaw Survival and Proliferation of Friable and Embryogenic Callus in *Hevea brasiliensis* by Liquid Nitrogen Vitrification Cryopreservation Method

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ABSTRACT

Friable and embryogenic calli could be induced from the inner integument of the rubber tree clone Reyan 88-13. And they had been proliferated and maintained for more than 2 years. Somatic embryos and further regeneration plantlets had been obtained from these calli. In order to preserve these calli for a long time and maintain their genetic characters, a liquid nitrogen vitrification cryopreservation method had been studied with these calli as the material. These calli were inoculated on the pre-culture medium, and had been cultured for 2-3 days in the dark, the temperature was 25-28℃. Then the calli were immersed for 10-30 min in the 60% PVS_2 solution at room temperature. After dehydration treatment, these calli were immersed for 20-40 min in PVS_2 solution (28%-32% glycerol + 12%-18% hexanediol + 12%-18% dimethyl sulphoxide +136.9g/L sucrose) at 0℃. Then the former PVS_2 solution was removed, and new one was added. Afterward, these calli were thrown into liquid nitrogen (-196℃) rapidly. The recovery of the calli was to take out of them from the liquid nitrogen quickly, immersed them in water for 2-3 min in which the temperature was 40℃. After thaw and rinse, these calli were transferred on the subculture medium, and was cultured for 2-3 days in the dark, the temperature was 25-28℃. Then they were transferred on the fresh subculture medium and were cultured for 30 days. Most of the cryopreserved calli turned brown and died. But some cell survived. So 30 days later, some tiny calli emerged, and grew. And they could be proliferated in the further subculture cycles. The post-thaw and survival calli could be used to induce somatic

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embryogenesis and further plant regeneration. The present results of the studies had the advantages that the experimental procedure was not complex, expensive apparatus could be avoided, repeatability and effect was good. So this method would be useful for long-term preservation of valuable germplasm materials, and would be beneficial for tissue culture and genetic transformation studies in *Hevea brasiliensis*.

橡胶树易碎胚性愈伤组织液氮玻璃化超低温冷冻保存冻后成活和增殖

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摘要

用橡胶树品种热研88-13的内珠被可诱导出易碎的胚性愈伤组织。它们增殖和继代培养了两年多长的时间。从这些愈伤组织诱导出了体细胞胚，并得到再生植株。为了长期保存这些愈伤组织，维持它们的遗传性状，以这些愈伤组织为材料，研究了液氮玻璃化超低温冷冻保存方法。然后在室温下用60%的PVS_2处理花药愈伤组织10～30分钟；脱水处理后，在0℃下用PVS_2处理20～40分钟 (28%-32% 甘油+ 12%-18% 己二醇+ 12%-18% 二甲亚砜+136.9g/L 蔗糖)，移去PVS_2原液，再加入新的PVS2，在液氮中保存。花药愈伤组织的复苏是在40℃水浴中迅速浸泡2~3分钟，化冻洗涤后，在25~28℃的继代培养基上暗培养2~3天，再继代培养30天。大多数这些经过超低温冷冻保存的愈伤组织褐化和死亡，但一些细胞存活。30天后，一些小愈伤组织颗粒出现和生长，可以在后续的培养中增殖。诱导胚状体和诱导成熟胚状体分化后可获得再生植株。本研究所涉及的液氮玻璃化超低温保存方法具有设备简便、实验程序简化、效果和重复性好等优点，可长期保存橡胶树有价值的种质资源，可为橡胶树组织培养和遗传转化研究提供有利条件。

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